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The Effects of Environmental Temperature on Locomotor Performance and Growth Patterns in Spotted Salamander, Ambystoma Maculatum

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THE EFFECTS OF ENVIRONMENTAL TEMPERATURE ON LOCOMOTOR PERFORMANCE AND GROWTH PATTERNS IN SPOTTED SALAMANDER, *AMBYSTOMA MACULATUM*

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by

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DEDICATION

To Maria de Fatima Rodrigues Almeida, Bertran de Almeida, and Toby Dragon.

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ABSTRACT

THE EFFECTS OF ENVIRONMENTAL TEMPERATURE ON LOCOMOTOR PERFORMANCE AND GROWTH PATTERNS IN SPOTTED SALAMANDER, *AMBYSTOMA MACULATUM*

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Variation in temperature has a profound effect on many aspects of an animal's physiology, behavior, and performance capacities. Although animals are capable of coping with a range of temperature, they are adapted to specific boundaries of temperature. In an era of global climate change, it is fundamental to comprehend how organisms will react in relation to temperature-related stress and how warmer environmental temperature will affect whole organism performance, as these traits are often crucial to survival.

In this study, I examined the effects of temperature on time to hatching period, body length, and larval growth rate. Specifically, I address the following two questions. First, does an increase in temperature affect the duration of time to hatching period? Second, do temperature and the duration of the time to hatching period affect body length at the time of hatching, subsequent growth rate? Furthermore, I investigate the effects of temperature on larval locomotor performance by examining whether or not temperature can result in any impairment of locomotor performance variables (velocity and

acceleration). Specifically, I wish to address the following question, does an increase in environmental temperature affect both larval maximum and average velocity and acceleration? In order to answer such questions I raised one egg cluster of Spotted Salamander, *Ambystoma maculatum*, in two different temperatures (15°C and 21°C). I maintained both the eggs and the resulting larvae in these different temperature regimes until the larvae had reached two weeks of age. I then examined the effects of temperature on body length, growth rate, and locomotor performance.

I found that temperature does not have a direct significant effect on body length in *A. maculatum*. However, I found that temperature has a significant effect on the length of time to hatching period and that the length of time to hatching period is directly correlated to body length. I also found that temperature does not have a significant effect on larval velocity but does have a significant effect on larval acceleration.

I argue here that an increase in the mean environmental temperature could result in a decrease in locomotor performance and consequent higher predation susceptibility.

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CHAPTER 1

THE EFFECTS OF ENVIRONMENTAL TEMPERATURE ON GROWTH PATTERNS IN SPOTTED SALAMANDERS, *AMBYSTOMA MACULATUM*

1.1 Introduction

Temperature is one of the most important abiotic environmental factors as changes in environmental temperature, and consequently changes on internal body temperature may greatly increase mortality, especially in ectothermic species, which have limited ability to regulate their internal temperature. Environmental temperature can vary dramatically over daily, seasonal, or developmental time scales, eliciting responses at levels ranging from the gene to the whole organism (Johnston and Temple 2002). These responses can include changes on the animal's physiology, behavior, and performance capacity as a compensatory response to the exposure of different temperature treatments. For instance, in ectotherms, the rising or falling of body temperature leads to an increase or decrease in metabolic rate reflecting changes in the balance between consumption and generation of Adenosine-5'-triphosphate (ATP) (Johnston and Temple 2002). Ectothermic animals, although often capable of coping with a range of environmental temperature, are adapted to specific temperature boundaries and prolonged exposure to thermal conditions outside the lower or upper tolerance limit can often be detrimental, especially during development. For example, Anderson (1972) demonstrated that five species of *Ambystoma* salamanders were adapted to specific temperature ranges and that the exposure of these animals to temperatures outside these ranges during the developmental stage resulted in extremely high mortality. For example, there was a mortality rate of approximately 90% for *A. sigillatum* and 50% for *A. croceum*.

Among ectotherms, amphibians also present limited physiological abilities to control their body temperatures thus they are highly susceptible to temperature fluctuations in their environments. Given that most climate models predict that a long-term build-up of greenhouse gases is likely to increase surface air temperature an average of 1.5°C to 4.5°C (Melillo et al. 1993) in most regions of the world over the next several hundred years, it is useful to assess the effects of such temperature changes on ectotherms. In addition, vernal pond breeding amphibians could be one of the most notably affected ectotherms because these animals spend the first crucial months of life as larvae confined to small bodies of water, which are often susceptible to large fluctuations in temperature.

Temperature affects many morphological traits during development (Brana and Ji 2000), but total body length, here defined as the measurement from the tip of the head to the tip of the tail, is an important trait for several species of salamanders because it has implications for a salamander's survival. For example, larval body length at the time of hatching together with developmental growth rate determines body length and age at metamorphosis (O' Laughlin and Harris 2000). Body length at metamorphosis influences females' age and size at the time of first reproductive episode; with older, larger females being able to produce larger clutches and sometimes-larger individual eggs (Semlitsch and Gibbons 1990). Larval body length could also have an indirect effect on larval survival, since larger individuals have enhanced locomotor performance, providing a potential advantage during predatory attack (Batty and Blaxter 1992). In addition, the length of juvenile period and body length at first reproduction are critical life history traits that have a strong influence on fitness (Gotthard 2000). A short juvenile period is in

some cases beneficial because it reduces the risk of predation before reproduction but it also results in a short generation time (Gotthard 2001). On the other hand, larger adult body is often correlated with higher female fecundity and competitive ability on both males and females (Gotthard 2001). Growth rate, which describes the relationship between body length and the length of the juvenile period (Arendt 1997), plays a pivotal role because it determines the position of an individual along the spectrum between adult body length and time needed to reach adulthood. Although most aquatic amphibians may exhibit the same degree of correlation between body length, age, and environmental temperature (Angilletta et al. 2004), there are very few studies on the possible effects of environmental temperature on body length and the subsequent influences on size-related traits, with most of these examples concentrating only on marine organisms.

In this study, I used the Spotted Salamander (*Ambystoma maculatum*) to investigate the possible effects of increased environmental temperatures on larval body length. Spotted Salamanders range over much of North America, from Prince Edward Island to south-central Ontario, and south to Georgia and eastern Texas (Retrieved from http://animaldiversity.ummz.umich.edu/site/accounts/information/Ambystoma_maculatum.html). Rain and warming temperatures elicit migrations of adult salamanders to breeding ponds in winter or early spring (Grace and Church 2003). At this time, Spotted Salamanders tend to lay clusters of eggs in mid-April with approximately 250 eggs per cluster. They have a fast rate of development in which the larva hatches approximately in one to two months after oviposition and metamorphosis begins 2 to 4 months after hatching (States et al. 1988). In Massachusetts, the average period of time the animal spends in a vernal pond is approximately 98 days (States et al. 1988).

With this study, I extend the previous work on amphibian body length by examining whether environmental temperature can affect time to hatching, body length at the time of hatching, and growth rate. Specifically, I address three questions. First, does an increase in environmental temperature affect the time to hatching? Second, do environmental temperature and the duration of time to hatching period affect body length at the time of hatching and subsequent growth rate? Third, if environmental temperature does affect body length, what is the growth pattern? Is there just an increase in tail length or an increase the length of other body segments? I predict that environmental temperature closer to the upper limit of the spotted salamander tolerance range will decrease the duration of time to hatching and produce larvae that are both smaller and less developed than the larvae raised at lower environmental temperature.

1.2 Methods

1.2.1 Data collection

One egg mass of *Ambystoma maculatum* was obtained from a vernal pond in Hampden County, Massachusetts, on March 31, 2009. I obtained only one egg cluster in order to decrease genetic differences between the two treatment groups. I brought the egg cluster to the Irschick lab at the University of Massachusetts Amherst and once in the lab, I divided the egg mass into six small egg clusters (approximately 20 eggs in each small cluster). I kept each cluster in separate plastic containers that were each 9.5 cm tall and had a diameter of 15 cm. I filled the containers with a water mixture (120 ml of reverse osmosis water with approximately 0.012g of Kent® reverse osmosis reclaim chemistry mixture), which I changed daily. I randomly assigned each of the six clusters into two temperature treatments. I kept three small egg clusters (approximately a total of 60 eggs)

in a cold room at a temperature regime of 15°C (± 1°C) while I kept the three other small egg clusters (approximately a total of 60 eggs) in an incubator at a constant temperature of 21°C. I chose these experimental temperatures based on the temperature tolerance range for *A. maculatum.* The lower temperature tolerance is approximately 6°C and the maximum temperature tolerance is approximately 24°C (Moore 1939). I chose 15°C as the lower temperature group because 15°C is closer to the average mean environmental temperature (9.7°C) for the entire developmental period but not above the possible average mean temperature experience in a single day (approximately 23°C) in the region where I collected the egg mass (Figure 5). In addition, I chose 21°C for the individuals raised at higher environmental temperature because 21°C is not a temperature above *A. maculatum* high tolerance boundary (24°C) but it is a temperature very close to it.

Once the egg mass was collected, two HOBO pendant loggers® (Onset Computer Corp, Pocasset, MA) were placed in the vernal pond on April 8 2009. The HOBO pendant loggers® measured the temperature of the vernal pond every 15 minutes for a period of 12 weeks. The first HOBO pendant logger®(1) was placed at the exact place where the egg cluster was collected (deep water, approximately 0.5m) and the second HOBO pendant logger®(2) was placed at the edge of the vernal pool (shallow water). I used the data collected by the HOBO pendant loggers® to analyze and compare the differences in temperature experienced by the experimental population in my study to the natural temperature that this egg cluster and larvae would have experienced in the field.

Once the larvae started to hatch, I kept the hatchlings in individual plastic containers that were 9.5cm tall and 7.5cm wide. I changed the water in each container daily and -fed each salamander larva with frozen *Daphnia* every other day as soon as

they hatched. I took daily pictures of the larvae using a camera (Nikon model coolpix 995) attached to a dissecting scope. In order to do so, I placed each larva in a Petri dish with approximately 20 ml of water. I put a metric paper under the Petri dish, to provide a scale to measure the larva's body length. I used the pictures to measure larval body length at the time of hatching and to measured body length for a period of 20 to 35 days after hatching using the computer program ImageJ (version 6.0, Research Services Branch, National Institute of Mental Health, Bethesda, Maryland). I used the data on body length for only the first 20 to 35 days after hatching because after a period of 35 days most larvae in the 21°C treatment group had died. I then calculated growth rate for the first 14 days after hatching. I only used the data for the 14 days because at that time I transferred the larvae to cold room at 15°C in order to acclimate for the swimming trials. In addition, I recorded detailed notes on the day of hatching of each larva.

1.2.1.1 Statistical Analysis

I used ANOVA to test the effects of environmental temperature on time to hatching period. I designated environmental temperature as the independent variable and the time to hatching period as the dependent variable. I used ANCOVA to test the effects of environmental temperature on body length at the time of hatching and at two weeks after hatching. For this test, I designated environmental temperature as the independent variable, time to hatching period as the covariate and body length at the two stages as dependent variables. I used ANOVA to test the effects of environmental temperature on growth rate. I set environmental temperature as the independent variable and growth rate as the dependent variable. I conducted all tests using JMP statistical software (version 8.0, SAS Institute, Cary, NC).

1.3 Results

Environmental temperature did not have a statistically significant effect on body length at the time of hatching (Figure 1; Table 1) or on body size 20 to 35 days after hatching (Figure 2; Table 2). However, environmental temperature did have a statistically significant effect on growth rate over a period of 14 days (Table 3). In addition, environmental temperature had a statistically significant effect on time to hatching. The mean time to hatching period for individuals at the 15°C treatment group was 27 days while the mean time to hatching for individuals at the 21°C treatment group was 18 days. Thus, the mean time to hatching for individuals at the 15°C treatment group was in average 9 days longer than the time to hatching for individuals at the 21°C treatment group (Figure 3). Although environmental temperature did not have a significant effect on body length, time to hatching had a significant effect on both body length at the time of hatching (Table 1) and body length 20 to 35 days after hatching (Table 2). Within treatment groups, I observed that the time to hatching is directly correlated to body size at the time of hatching.

Time of hatching and the specific hatching date in the lab for each salamander larvae is provided on appendix A and the body size at the time of hatching for each salamander larvae is provided on appendix B. In addition, the data from HOBO pendant loggers®(1) is proved on Table 4 and the data from HOBO pendant loggers®(2) is provided on Table 5.

1.4 Discussion

In this study, I found that environmental temperature does not have a significant effect on larval body length at the time of hatching for the salamander species

Ambystoma maculatum. I also found that environmental temperature does have an effect on larval growth rate for this species during a period of 14 days. Finally, I found that environmental temperature has a significant effect on the time to hatching, by decreasing it by an average of 9 days. When analyzing the effects of time to hatching on body length, I also found that, within treatment groups, time to hatching is directly correlated to body length at the time of hatching.

Body length at maturity for a particular individual is constrained by its larval growth trajectory, of which growth rate is a key element (Gotthard 2000). For ectotherms, temperature strongly affects differences in body length, rates of growth, and development (Sibly and Atkinson 1994). Therefore, an increase in temperature tends to increase overall growth rate in ectotherms. In this study, I found that an increase of 6°C is enough to observe a slight difference in growth rate between treatment groups. In addition, it is important to consider that I calculated growth rate for only a period of 14 days whereas most studies that observe growth rate tend to calculate growth rate for a longer period of time. The results of this study show that even during a small period of time a slight increase in environmental temperatures can still have an effect on growth pattern, in this case growth rate. Interestingly, some growth models have assumed that larvae tend to grow to the largest body length possible in the shortest period of time but a growing body of evidence suggests that individuals often grow at a lower rate than they are physiologically capable of (Gotthard 2001). These studies suggest that high growth rates might be associated with certain fitness costs (Gotthard 2001), including physiological, developmental, and ecological costs. For example, empirical evidence has demonstrated that some of the costs of rapid growth include increased fluctuation asymmetry, reduced

immune capacity, and reduced ability to responds to environmental stress (Arendt 1997). In future studies, it would be interesting to investigate if these costs are observed in this species of salamander or if higher environmental temperatures would increase growth rate to a certain extent without significant effect on body morphology or body immune response.

In this study, environmental temperature could only have affected body length if temperature somehow affected the conversion rate of egg yolk into body tissue. I found that both groups of individuals had similar body length at the time of hatching and similar body length throughout the 20 to 35 days after hatching, with the exception of only two individuals in the 21°C treatment group, which had extremely small body lengths. Larval body length at the time of hatching is constrained by the amount of egg yolk and the conversion rate of egg yolk to body tissue. Since temperature did not affect those two factors, there was no correlation between environmental temperature and body length for either treatment group. However, when comparing the effects of time to hatching on body length within groups, the results show that time to hatching has an effect on body length. In both treatment groups, body length at the time of hatching and body length 20 to 35 days after hatching was correlated to time to hatching period. This correlation between body length and age is perhaps because individuals that hatch later are allowed more time to convert the initial large amounts of yolk into tissue. In addition, the increase in body length observed within individuals in the 15°C treatment group was throughout the body. The increase in body length was presented in the head, body segment, and tail.

The length of time to hatching can be greatly influenced by certain conditions such as depth and location of egg cluster within the vernal pond and the mean

environmental temperature of the water. Some studies have shown that an increase in environmental temperature exponentially decreases the length of time to hatching in some marine ectotherms such as *S. serrata* (Hamasaki 2003), *S. paramamosain* (Hamasaki 2002). In the case of *A. maculatum*, the time to hatching varies depending on the region but in Massachusetts, this species usually has a time to hatching period of 39 days (States et al. 1988). Individuals raised at a lower environmental temperature had on average a time to hatching period 9 days longer than individuals that developed at the higher temperature. However, body length is a complex trait influenced by several factors such as length of time to hatching, growth rate, food availability, etc. The results on the effects of time to hatching on body length within treatment groups could be interpreted as a possible way of how environmental temperature could come to affect body length at the time of hatching. The length of time to hatching could decrease to a point in which it could cause larvae not to have enough time to convert all the egg yolk available to body tissue. This causes larvae to hatch at a smaller body length. However, with the present results on both growth patterns and growth rate, it is likely that each one will cancel the other out and we will not see a drastic difference in body length.

Studies on ectotherms have previously focused on the behavior and performance capacity at optimal environmental temperatures. However, with the increase of data available on the possible outcomes of global climate change, researchers have focused on what exactly an increase or decrease in the mean environmental temperature means to an ectotherm in several stages of its life. The results from this study show that environmental temperature did not directly affect salamander larval body length. However, there is still a need for more studies on this subject in order to have a more

complete assessment of the possible effects of an increase in environmental temperature will have on this species, especially on the effect of environmental temperature on post-metamorphosis body size.

Table 1: ANCOVA results for the effects of temperature and time to hatching period on body length at the time of hatching.

Source	Df	SS	F	P
Temperature	1	1.128	1.152	0.289
Time to hatching	1	4.059	4.143	0.048
Error	41	40.16		

Table 2: ANCOVA results for the effects of temperature and time to hatching period on body length at 20 to 35 days after hatching.

Source	Df	SS	F	P
Temperature	1	0.739	2.46	0.124
Time to hatching	1	6.331	21.13	0.000
Error	41	12.28		

Table 3: ANCOVA results for the effects of temperature and time to hatching period on growth rate for a period of 14 days.

Source	Df	SS	F	P
Temperature	1	0.000	4.265	0.045
Time to hatching	1	0.000	0.359	0.552
Error	43	0.000		

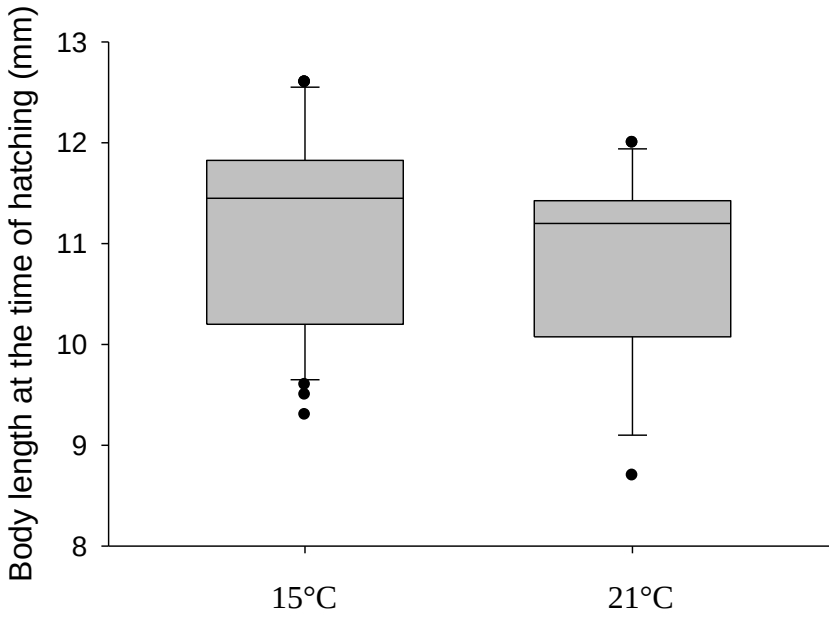

Figure 1. The effects of different temperature treatments (15°C and 21°C) on body length at the time of hatching for the salamander species *Ambystoma maculatum*. Box and whisker plots for mean initial body length. Boxes represent the median, lower and upper quartiles. Whiskers represent the 10^{th} and 90^{th} percentile. Outliers are represented by individual points. N=34 for 15°C treatment group and N=22 for 21°C treatment group.

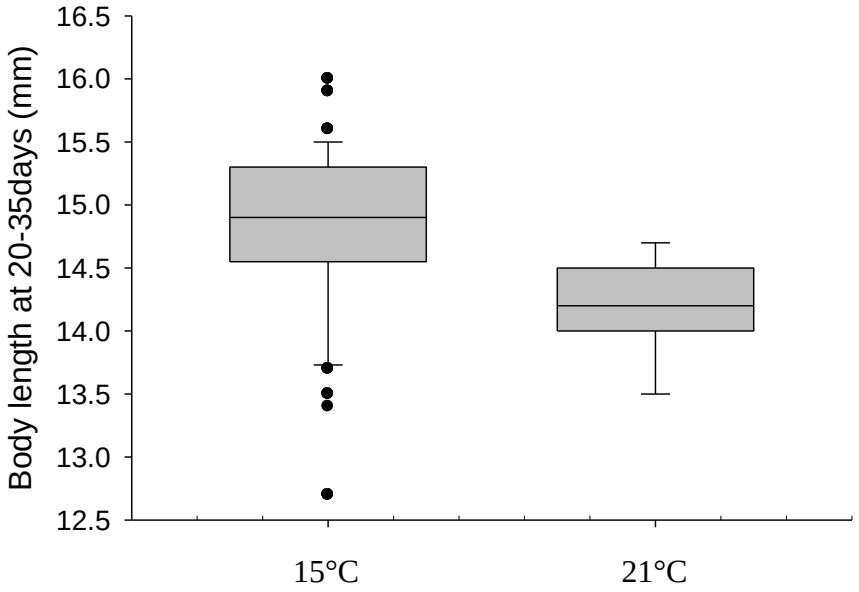

Figure 2. The effects of different temperature treatments (15°C and 21°C) on body length at 20 to 35 days after hatching for the salamander species *Ambystoma maculatum*. Box and whisker plots for mean initial body length. Boxes represent the median, lower and upper quartiles. Whiskers represent the 10^{th} and 90^{th} percentile. Outliers are represented by individual points. N=34 for 15°C treatment group and N=22 for 21°C treatment group.

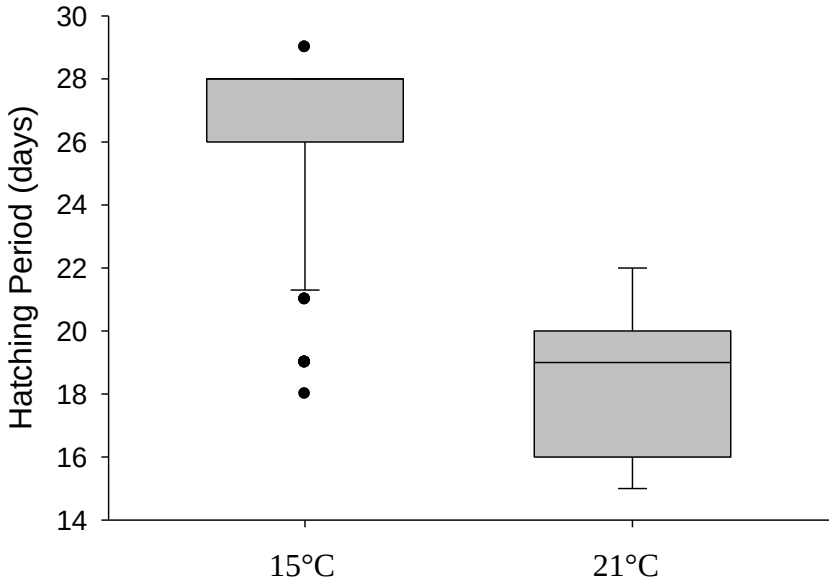

Figure 3. The effects of different temperature treatments (15°C and 21°C) on the length of time to hatching period for the salamander species *Ambystoma maculatum*. Box and whisker plots for mean length of time to hatching. Boxes represent the median, lower and upper quartiles. Whiskers represent the 10^{th} and 90^{th} percentile. Outliers are represented by individual points. N=34 for 15°C treatment group and N=22 for 21°C treatment group.

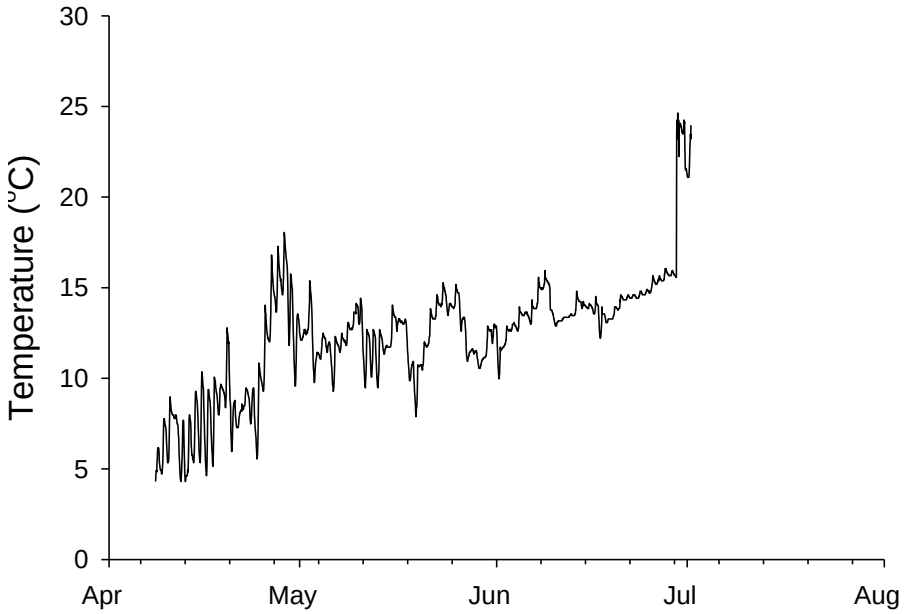

Figure 4. Site variation in mean environmental temperature for the period of three months from HOBO logger (1). The data for this graph was based on the data collected by the HOBO logger®(1) placed in deep water (approximately less than 1meter deep).

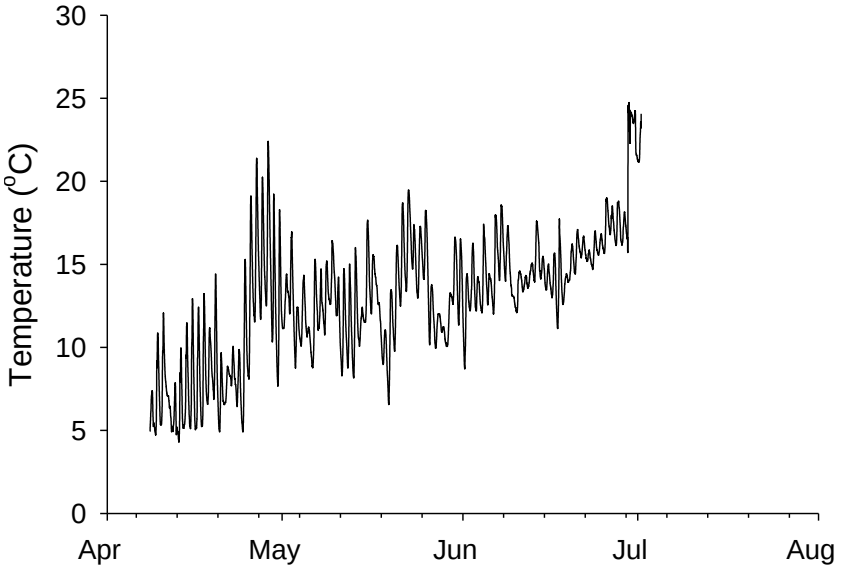

Figure 5. Site variation in mean environmental temperature for the period of three months from HOBO logger (2). The data for this graph was based on the data collected by the HOBO logger®(2) placed in deep water (approximately 5 meters deep).

CHAPTER 2

THE EFFECTS OF ENVIRONMENTAL TEMPERATURE ON LARVAL LOCOMOTOR PERFORMANCE IN SPOTTED SALAMANDERS, *AMBYSTOMA MACULATUM*

2.1 Iintroduction

The ability of an organism to perform key tasks, such as capturing prey or escaping predators, may have far-reaching effects on organismal survival, reproduction, and growth (Irschick and Losos 1998). For example, the inability of a larva to swim at a sufficient velocity and acceleration could have negative consequences on its survival or on its ability to catch prey. Therefore, locomotor performance could be essential for an organism's early survival. Environmental temperature has been shown to have profound consequences for whole-animal performance (Watkins 2000), which here is defined as a quantitative measure of how well an organism accomplishes some ecologically relevant task (Irschick et al. 2009). In many cases, there exists an optimal range of temperatures for maximum organismal performance (Bennett 1990). For example, maximum locomotor performance of vertebrates typically peaks at intermediate temperatures and decreases substantially above and below the "optimal" temperature or plateau (Bennett 1990). In addition, ecological studies have also linked variation in preferred body temperature with variation in some aspects of locomotor performance, such as maximal velocity (Bergmann and Irschick 2006). Endothermic homeotherms avoid these perturbations by maintaining their internal body temperature within the optimal range at great energetic cost, but poikilotherms may be subjected to major daily and seasonal fluctuations in body temperature, and these changes in temperature can dramatically

decrease maximum speed, velocity, and endurance of ectothermic species (Bennett 1990). For example, Stevenson et al. (1985) showed that garter snakes, *Thamnophis elegans*, suffer a considerable decrease in crawling and swimming speed during the night when temperature is outside their optimal range. Thus, we can conclude that body temperature has profound effects on the performance and consequently on fitness of ectotherms (Martin and Huey 2007) because temperature-induced effects on locomotor performance can influence the capability of ectotherms to escape from predators and to capture prey (Li and Wang 2005). Hence, we can hypothesize that the ability of individuals to survive may depend to some extent on the temperature regimes at which they develop.

Organismal performance is not solely the result of an organism's genotype and post-hatching environmental variables but also the result of the environmental variables experienced during embryonic formation. In reptiles, the thermal environments that hatchlings experience during development will affect various phenotypic traits including body length, length of incubation, hatching success, oxygen consumption, blood pH, and sex determination (Brana and Ji 2000). There is growing evidence demonstrating that environmental conditions during embryogenesis can affect an animal's phenotype (Brana and Ji 2000), which could lead to changes in performance ability, such as swimming, jumping, and crawling performance. The effects of environmental temperature on performance are especially important because perturbations that occur early in ontogeny, when developmental "decisions" are being made, can have long-lasting consequences throughout the individual's life span (Watkins 2000). A crucial issue when studying the effects of environmental temperature during embryogenesis is to identify if there is a shift

in the hatchling's thermal optimum for performance or, if there is a permanent alteration in organismal performance capacity. Although there has been extensive work on how environmental temperature affects the performances during the adult stage, we only have limited knowledge about the effects of environmental temperature on larvae and juveniles. Given the drastic effects of temperature on the embryogenesis of reptiles, there is a need to investigate the effects of environmental temperature in other ectothermic groups. In this study, I will try to answer the previous two questions by measuring the effects of environmental temperature on velocity and acceleration, two measures of locomotor performance that are particularly important to small aquatic vertebrates.

In this study, I focus on larval swimming performance because it is during this life stage that the highest escape performance is expected to occur unless developmental or functional constraints interfere (Landberg and Azizi 2009). Peak aquatic escape performance in pond-breeding amphibians is expected to develop early in the larval period and then decrease with the increase in body size and the loss of the tail (Landberg and Azizi 2009). In addition, I consider swimming performance important because the outcome of predatory encounters is a major determinant of survival in both predator and prey species. Therefore, variables that affect the ontogeny of swimming escape performance will have direct fitness consequences for this group of animals. In this study, I define swimming performance as both larval velocity and acceleration. Although both maximum speed and acceleration are potentially important for small ectotherms in natural situations, rapid acceleration is likely to be especially important for species that must flee, sit, and wait for predators, such as aquatic insects.

In this study, I used the Spotted Salamander (*Ambystoma maculatum*) to investigate the possible effects of increased environmental temperatures on maximum and average values of velocity and acceleration in larval salamanders. Specifically, I tested the hypothesis that an increase in environmental temperature affects larval maximum and average velocity and acceleration. I predict that individuals raised at environmental temperature closer to their upper tolerance limit range will have reduced maximum and average acceleration and velocity than the larvae raised at lower environmental temperatures. The results of this study will provide insight into the effects of microhabitat temperature changes of this species of salamander, *A. maculatum*, and could help us better understand and predict the possible effects that global climate change could have on this species and other ectotherms.

2.2 Methods

2.2.1 Data collection and animal care

One egg mass of *Ambystoma maculatum* was obtained from a vernal pond in Hampden County, Massachusetts, on March 31, 2009. I obtained only one egg cluster to decrease genetic differences between the two different treatment groups. I brought the egg cluster to the Irschick lab at the University of Massachusetts Amherst and once in the lab, I divided the egg mass into six small egg clusters (approximately 20 eggs in each cluster). I kept each cluster in separate plastic containers that were each 9.5 cm tall and had a diameter of 15 cm. I filled the containers with a water mixture (120 ml of reverse osmosis water with approximately 0.012g of Kent® reverse osmosis reclaim chemistry mixture), which I changed daily. I randomly assigned each of the six clusters into two temperature treatments. I kept three small egg clusters (approximately 60 eggs) in a cold

room at a temperature regime of 15°C (± 1°C) while I kept the other three small egg clusters (approximately 60 eggs) in an incubator at a constant temperature of 21°C. I chose the experimental temperatures based on the temperature tolerance range for *A. maculatum*, which are lower temperature tolerance approximately 6°C and maximum temperature tolerance 24°C (Moore 1939). I chose 15°C as the lower temperature group because 15°C is closer to the average mean environmental temperature (9.7°C) for the entire time to hatching period but not above the possible average mean temperature experience in a single day (approximately 23°C) in the region where I collected the egg mass (Figure 1). I then chose 21°C for the individuals raised at higher environmental temperature because 21°C is not a temperature above *A. maculatum* high tolerance boundary (24°C) but it is a temperature very close to it (Moore 1939).

Once the egg mass was collected, two HOBO pendant loggers® (Onset Computer Corp, Pocasset, MA) were placed in the vernal pond on April 8 2009. The HOBO loggers® measured the temperature of the vernal pond every 15 minutes for a period of 12 weeks. The first HOBO logger® was placed at the exact place where the egg cluster was collected (deep water, approximately 0.5m) and the second HOBO logger® was placed at the edge of the vernal pool (shallow water). I used the data collected by the HOBO loggers® to analyze and compare the differences in temperature experienced by the experimental population in my study to the natural temperature that this egg cluster and larvae would have experienced in the field.

Once the larvae started to hatch, I kept each hatchling in individual plastic containers that was 9.5cm tall and 7.5cm wide. I changed the water in each container daily and -fed each salamander larva with frozen *Daphnia* every other day as soon as

they hatched. I took daily pictures of the larvae using a camera (Nikon model coolpix 995) attached to a dissecting scope. In order to do so, I placed each larva in a Petri dish with approximately 20 ml of water. I put a metric paper under the Petri dish, to provide a scale to measure the larva's body length. I used the pictures to measure larval body length at the time of hatching during the first 21 to 35 days after hatching and to calculate growth rate during the first 14 days after hatching, using the computer program ImageJ (version 6.0, Research Services Branch, National Institute of Mental Health, Bethesda, Maryland). I used the data on body size for only the first 20 to 35 days after hatching because after a period of 35 days most larvae in the 21°C treatment group had died. These data were then used to compare the rate of development between the two temperature treatments. In addition, I recorded detailed notes on the day of hatching of each larva.

2.2.1.1 Swimming trials

I conducted swimming trials three weeks after larvae had hatched. One week before I started to conduct the swimming trials, I transferred the larvae raised at 21°C to the cold room at 15°C (± 1°C). It was at this cold room that I conducted all swimming trials. I did this to allow larvae to acclimate to a colder temperature before trials began and to ensure that the differences in swimming performance were not the result of larvae swimming in warmer water. Each swimming trial consisted of three swimming events for each salamander larvae, which I ran once a week for a period of three weeks. I transferred each larva into a small plastic container (performance arena) with approximately 800 ml of water and a metric scale on the bottom. I allowed each salamander to acclimate for two minutes. After two minutes, I touched the tip of the salamander's tail with a probe to

induce escape behavior. I filmed all trials using a camera NAC HSV-500 high-speed video system operating at 500 images per second. I attached the camera to a tripod 60 cm above the performance arena. After the trial, I placed the larvae back in their specific containers. Using the videos, I digitized one single point at the snout of larva using the Peak Performance software program (Motus Technologies, Denver, CO) to calculate maximum and average values of velocity and acceleration.

2.2.1.1.1 Statistical Analysis

I used the average value between the three trials as the value for average acceleration and velocity. I then used the maximum value within the three trials as the maximum velocity and acceleration. I used ANCOVA to test the effects of environmental temperature on maximum and average values of acceleration and velocity. For this test, I set environmental temperature as an independent variable, time to hatching period, body size at the time of hatching, and body size after 14 days as covariates and maximum and average values for velocity and acceleration as dependent variables. I conducted all tests using JMP statistical software (version 8.0, SAS Institute, Cary, NC).

2.3 Results

Environmental temperature did not have a significant effect on average velocity (Figure 2; Table 1). Individuals raised at an environmental temperature closer to their upper thermal tolerance limit (21°C) did not have on average lower or higher average velocity values than individuals did raised at 15°C. Similarly, environmental temperature also did not have a significant effect on maximum velocity (Figure 3; Table 2). Individuals raised at an environmental temperature at 21°C did not have lower or higher maximum velocity values than individuals raised at 15°C. Environmental temperature

had a significant effect on average acceleration (Figure 4; Table 3). Individuals raised at a environmental temperature closer to their upper thermal tolerance limit (21°C) had on average lower acceleration values than individuals raised at 15°C. Environmental temperature also had a significant effect on maximum acceleration (Figure 5; Table 4). Individuals raised at a environmental temperature of 21°C had lower maximum acceleration values than individuals raised at 15°C.

The time to hatching also did not have a significant effect on average velocity (Figure 6; Table 1) or on maximum velocity (Table 2). Time to hatching did not have a significant effect on either average acceleration (Table 3; Figure 7) or maximum acceleration (Table 4). Body size also did not have a significant effect on average velocity (Figure 8; Table 1) or on maximum velocity (Table 2). Body size did not have a significant effect on average acceleration (Figure 9; Table 3) and on maximum acceleration (Table 4).Body shape abnormality was presented in 3 out of 22 larvae (a total of 13.6% of the individuals) in the 21°C temperature treatment group (Figure 13).

The time of hatching and the specific hatching date in the lab for each salamander larvae is provided on appendix A and the body length at the time of hatching for each salamander larvae is provided on appendix B.

2.4 Discussion

In this study, I found that environmental temperature does not have an effect on average and maximum velocity for the salamander species *Ambystoma maculatum*, but higher environmental temperature caused a significant decline in maximum and average acceleration. In addition, I found that time to hatching and body length did not have an effected on either velocity or acceleration.

Locomotor performance is determined by underlying physiology, behavior, and the morphological structures during locomotion (Fitzpatrick et al. 2003). Consequently, temperature fluctuations that result in variability in physiology and or behavior may also influence the performance capacities that are directly linked to these functions. Speed and endurance, for example, may be limited by muscle contractile kinetics and oxygen transport capacities, both of which are temperature sensitive (Bennett 1990). One explanation for this locomotor pattern is that environmental temperature closer to the upper tolerance limit might be detrimental to the musculature in the larvae. Previous studies have shown that temperature can affect both muscle structure and enzymatic activity in some fish larvae (Watkins 2000). The acute exposure to increasing temperature causes a drastic decrease in ATPase activity, which in turn compromises muscle power output and consequently locomotor performance (Johnson and Bennett 1995). It is likely that this may be the case in *A. maculatum*. Since fast swimming confers a survival advantage to amphibian larvae in the presence of a predator (Watkins 2000), we can assume that larvae that are not capable of producing a fast burst of movement during predatory attacks will suffer higher mortality by predation. The results in this study suggest that if there is an increase in the mean environmental temperature in the field, it could potentially cause an increase in *A. maculatum* predation due to a decrease in its locomotor capacity. However, we are also assuming that an increase in the mean temperature will not have as a drastic effect on larger predator as it had on these larvae.

In this study, I did not detect any significant effect of environmental temperature on maximum and average velocity. Both temperature treatment groups had similar velocity values during the swimming trials. These results can lead us to conclude that in

relation to the ability to sustain long swim events, high thermal regimes (within a the tolerance limits of *A. maculatum*) do not have a detrimental effect in this aspect of locomotor performance. In addition, by using average velocity as an indicator of body condition, this study shows that although higher environmental temperatures might affect larval susceptibility to predation by affecting larval acceleration, the overall body condition of this animal was not drastically affected. However, the concept of larval body condition should also take into consideration other variables such as larval activity level and any type of body abnormality. In this study, I observed that higher environmental temperature resulted in individuals with lower activity levels compared to individuals raised at lower temperature treatment (personal observation).

The effects of environmental temperature on locomotor performance are independent of body length and time to hatching period. Failure to detect dependence between speed and body length is common when individuals are reared at constant temperature and measured at the same developmental stage because such experimental designs tend to result in little variation in body length (Watkins 2000). I argue that the lack of variation in body length and length of time to hatching period is not responsible for such observation on locomotor performance, as there was considerable variation in larval body length and length of time to hatching period.

Tadpoles of several species of frogs have shown to swim towards temperatures closer to their maximum performance both in the field and in the lab (Watkins 2000). Choosing the warmest temperature may maximize growth and developmental rates (Watkins 2000) but may also reduce body length and maximum swimming performance. The survival consequences of such behavioral thermoregulation depend on the selective

environment and having the ability to choose between environments that lie within thermal tolerance boundaries. However, for animals confined to small bodies of water the choice between suitable temperatures is limited. Because of this trade-off between developmental time and locomotor performance, the implications of an increase in mean environmental temperature on certain traits of swimming performance are expected to occur with greater frequency. Thus, the findings in this study are ecologically relevant since they bear on the possible outcomes of an increase in the mean average temperature over the next decades for this particular group of animals. Future research should focus on the effects of environmental temperature on locomotor performance after metamorphosis. This will allow us to know if the impairment on locomotor performance during larval stage will persist in the adult form. Furthermore, future studies should also investigate the actual mechanism that causes high development temperature to decrease average and maximum acceleration.

Table 4: ANCOVA results for the effects of temperature, time to hatching, and body length at the time of hatching on average velocity.

Source	Df	SS	F	P
Temperature	1	216.6	1.68	0.201
Time to hatching	1	0.393	0.003	0.956
Body length	1	63.03	0.490	0.488
Error	42	5404.67		

Table 5: ANCOVA results for the effects of temperature, time to hatching, and body length at the time of hatching on maximum velocity.

Source	Df	SS	F	P
Temperature	1	5.801	0.935	0.339
Time to hatching	1	17.95	2.89	0.096
Body length	1	0.088	0.014	0.906
Error	42	260.59		

Table 6: ANCOVA results for the effects of temperature, time to hatching, and body length at the time of hatching on average acceleration.

Source	Df	SS	F	P
Temperature	1	18637.5	10.43	0.002
Time to hatching	1	3731	2.08	0.156
Body length	1	711.4	0.398	0.531
Error	42	75040.9		

Table 7: ANCOVA results for the effects of temperature, time to hatching and body length at the time of hatching on maximum acceleration.

Source	Df	SS	F	P
Temperature	1	188145.3	7.23	0.01
Time to hatching	1	52639.8	2.02	0.162
Body length	1	13426.8	0.51	0.476
Error	42	1092549.1		

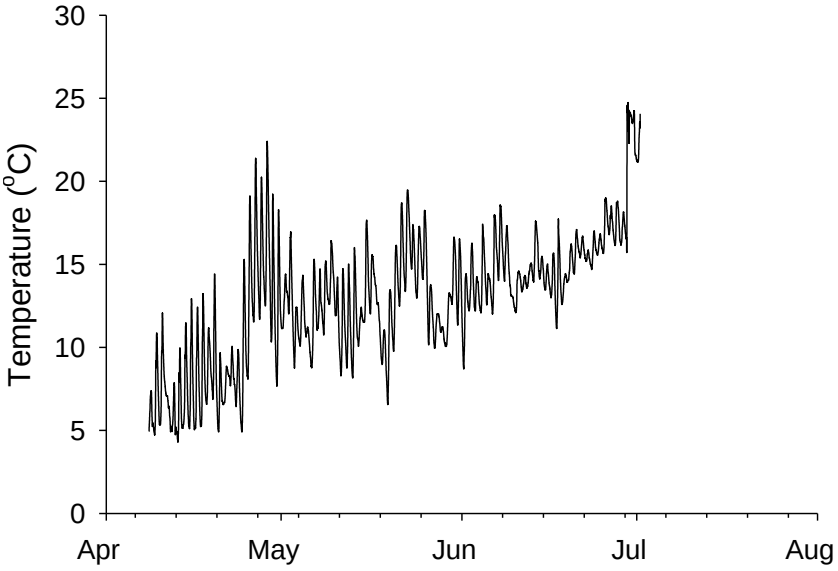

Figure 6. Site variation in mean environmental temperature for the period of three months from HOBO logger (2). The data for this graph was based on the data collected by the HOBO logger®(2) placed in deep water (approximately 5 meters deep).

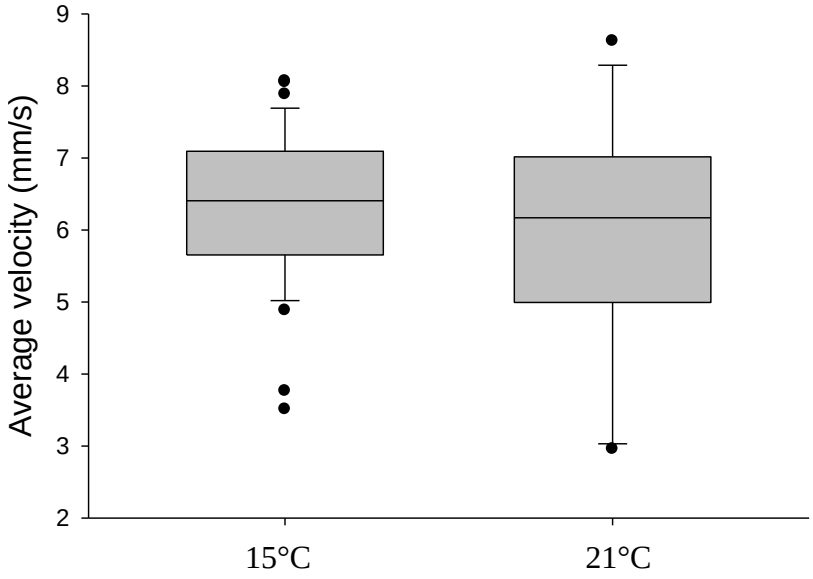

Figure 7. The effects of different temperature treatments (15°C and 21°C) on average velocity for the salamander species *Ambystoma maculatum*. Box and whisker plots for mean initial body size. Boxes represent the median, lower and upper quartiles. Whiskers represent the 10^{th} and 90^{th} percentile. Outliers are represented by individual points. N=34 for 15°C treatment group and N=22 for 21°C treatment group.

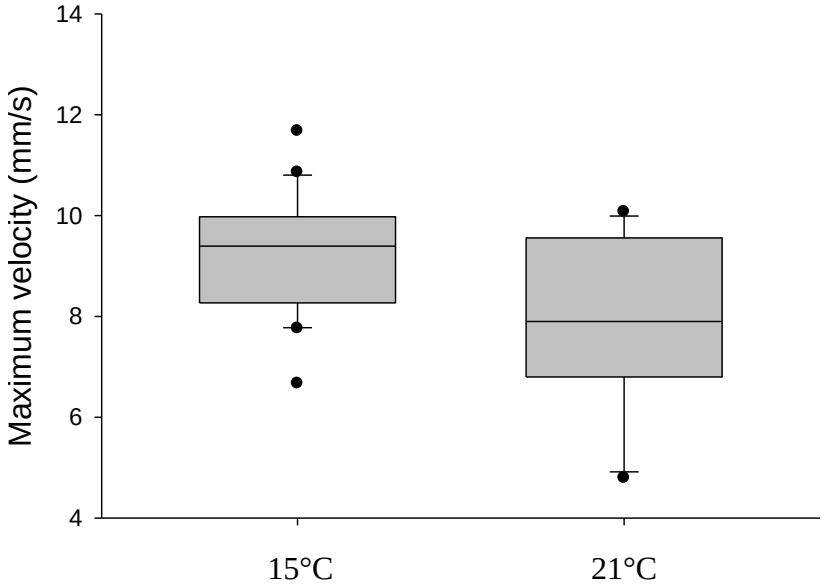

Figure 8. The effects of different temperature treatments (15°C and 21°C) on maximum velocity for the salamander species *Ambystoma maculatum*. Box and whisker plots for mean initial body size. Boxes represent the median, lower and upper quartiles. Whiskers represent the 10^{th} and 90^{th} percentile. Outliers are represented by individual points. N=34 for 15°C treatment group and N=22 for 21°C treatment group.

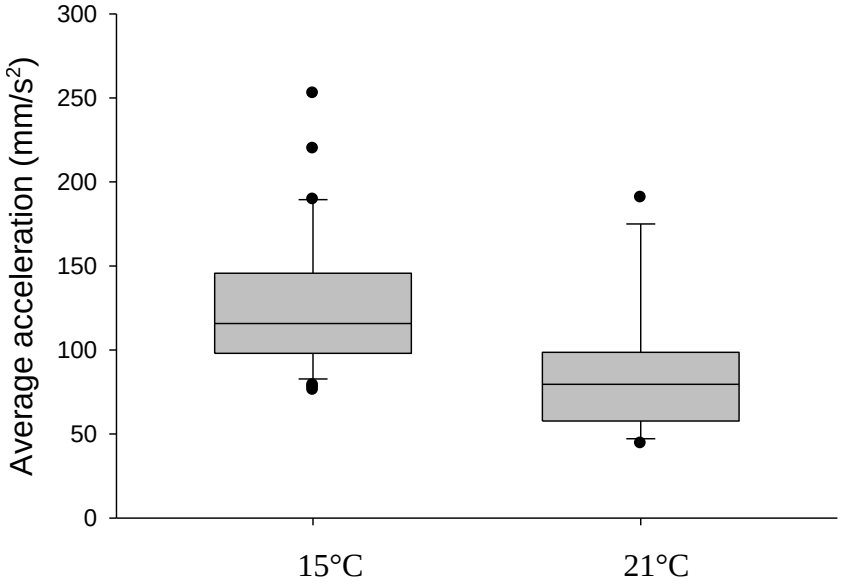

Figure 9. The effects of different temperature treatments (15°C and 21°C) on average acceleration for the salamander species *Ambystoma maculatum*. Box and whisker plots for mean average acceleration. Boxes represent the median, lower and upper quartiles. Whiskers represent the 10^{th} and 90^{th} percentile. Outliers are represented by individual points. N=34 for 15°C treatment group and N=22 for 21°C treatment group.

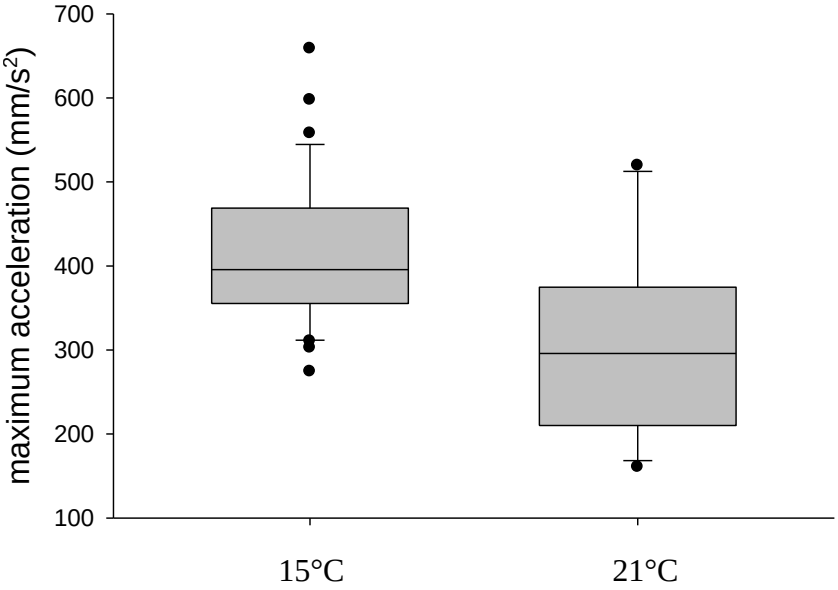

Figure 10. The effects of different temperature treatments (15°C and 21°C) on maximum acceleration for the salamander species *Ambystoma maculatum*. Box and whisker plots for mean acceleration. Boxes represent the median, lower and upper quartiles. Whiskers represent the 10^{th} and 90^{th} percentile. Outliers are represented by individual points. N=34 for 15°C treatment group and N=22 for 21°C treatment group.

APPENDIX A

THE HATCHING TIME (AGE) FOR EACH LARVA

Individual at 15°C	Hatching time	Individual at 21°C	Hatching time
1	19 (04/19/09)	1	13 (04/13/09)
2	20 (04/20/09)	2	14(04/14/09)
3	20 (04/20/09)	3	14(04/14/09)
4	22 (04/22/09)	4	16(04/16/09)
5	23 (04/23/09)	5	16(04/16/09)
6	25 (04/25/09)	6	16(04/16/09)
7	26 (04/26/09)	7	17(04/17/09)
8	26 (04/26/09)	8	17(04/17/09)
9	27 (04/27/09)	9	17(04/17/09)
10	27 (04/27/09)	10	17(04/17/09)
11	27 (04/27/09)	11	17(04/17/09)
12	27 (04/27/09)	12	18(04/18/09)
13	27 (04/27/09)	13	18(04/18/09)
14	27 (04/27/09)	14	18(04/18/09)
15	27 (04/27/09)	15	19(04/19/09)
16	27 (04/27/09)	16	20(04/20/09)
17	29 (04/29/09)	17	20(04/20/09)
18	29 (04/29/09)	18	20(04/20/09)
19	29 (04/29/09)	19	21(04/21/09)
20	29 (04/29/09)	20	21(04/21/09)
21	29 (04/29/09)	21	23(04/23/09)
22	29 (04/29/09)	22	26(04/26/09)
23	29 (04/29/09)		
24	29 (04/29/09)		
25	29 (04/29/09)		
26	29 (04/29/09)		
27	29 (04/29/09)		
28	29 (04/29/09)		
29	29 (04/29/09)		
30	29 (04/29/09)		
31	29 (04/29/09)		
32	29 (04/29/09)		
33	29 (04/29/09)		
34	30 (04/29/09)		

APPENDIX B

THE BODY LENGTH AT THE TIME OF HATCHING

Individual at 15°C	Body length (mm)	Individual at 21°C	Body length (mm)
1	10.8	1	10.3
2	9.7	2	10.6
3	10.6	3	11.2
4	10.7	4	11
5	10.1	5	10.1
6	9.6	6	11.4
7	9.7	7	11.8
8	10.5	8	11.2
9	10.1	9	12
10	10.2	10	12
11	9.5	11	11.5
12	10.2	12	11.6
13	9.3	13	11.2
14	10.7	14	11.4
15	12.1	15	11.4
16	11.4	16	11.3
17	12.6	17	10.9
18	12.5	18	10
19	11.8	19	9.6
20	11.7	20	9.1
21	12.6	21	8.7
22	12.4	22	9.1
23	11.8		
24	11.6		
25	11.8		
26	11.9		
27	11.5		
28	12.2		
29	12.4		
30	11.8		
31	11.6		
32	11.8		
33	11.9		
34	11.5		

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